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(54) Title: HUMAN CHEMOKINE BETA-13

(57) Abstract

The present invention relates to a CKbeta-13 (CKβ-13) protein which is a member of the chemokine family. In particular, isolated nucleic acid molecules are provided encoding the human CKβ-13 protein. CKβ-13 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of CKβ-13 activity. Also provided are diagnostic methods for detecting immune system-related disorders and therapeutic methods for treating immune system-related disorders.

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Human Chemokine Beta-13

Field of the Invention

The present invention relates to a novel human gene encoding a polypeptide which is a member of the chemokine family. More specifically, isolated nucleic acid molecules are provided encoding a human polypeptide named Human Chemokine Beta-13, hereinafter referred to as "CKβ-13." Polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of CKβ-13 activity.

Background of the Invention

Chemokines, also referred to as intercrine cytokines, are a subfamily of structurally and functionally related cytokines. These molecules are 8-10 kd in size. In general, chemokines exhibit 20% to 75% homology at the amino acid level and are characterized by four conserved cysteine residues that form two disulfide bonds. Based on the arrangement of the first two cysteine residues, chemokines have been classified into two subfamilies, alpha and beta. In the alpha subfamily, the first two cysteines are separated by one amino acid and hence are referred to as the "C-X-C" subfamily. In the beta subfamily, the two cysteines are in an adjacent position and are, therefore, referred to as the "C-C" subfamily. Thus far, at least nine different members of this family have been identified in humans.

The intercrine cytokines exhibit a wide variety of functions. A hallmark feature is their ability to elicit chemotactic migration of distinct cell types, including monocytes, neutrophils, T lymphocytes, basophils, and fibroblasts. Many chemokines have proinflammatory activity and are involved in multiple stpes during an inflammatory reaction. These activities include stimulation of histamine release, lysosomal enzyme and leukotriene release, increased adherence of target immune cells to endothelial cells, enchanced binding of complement proteins, induced expression of granulocyte adhesion molecules and complement receptors, and respiratory burst. In addition to their involvment in inflammation, certain chemokines have been shown to exhibit other activities. For example, macrophage inflammatory protein-1 (MIP-1) is able to suppress hematopoietic stem cell proliferation, platelet factor-4 (PF-4) is a potent inhibitor of endothelial cell growth, interleukin-8 (IL-8) promotes proliferation of keratinocytes, and GRO is an autocrine growth factor for melanoma cells.

In light of the diverse biological activities, it is not surprising that chemokines have been implicated in a number of physiological and disease conditions, including lymphocyte trafficking, wound healing, hematopoietic regulation and immunological disorders such as allergy, asthma and arthritis.

Members of the "C-C" branch exert their effects on the following cells: eosinophils which destroy parasites to lessen parasitic infection and cause chronic inflammation in the airways of the respiratory system; monocytes and macrophages which suppress tumor formation in vertebrates; T lymphocytes which attract T cells and basophils which release histamine which plays a role in allergic inflammation.

While members of the C-C branch act predominately on mononuclear cells and members of the C-X-C branch act predominantly on neutorphils a distinct chemoattractant property cannot be assigned to a chemokine based on this guideline. Some chemokines from one family show characteristics of the other.

The polypeptide of the present invention has the conserved cysteine "C-C" region, and has amino acid sequence homology to other known chemokines.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of the CKβ-13 polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 or the complete amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 97113 on April 28, 1995 . The nucleotide sequence determined by sequencing the deposited CKβ-13 clone, which is shown in Figure 1 (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 93 amino acid residues, including an initiation codon encoding an N-terminal methionine at nucleotide positions 1-3 .

The polypeptide of the present invention has amino acid sequence homology to known chemokines, including the conserved cysteine pattern characteristic of the beta subfamily of chemokines beginning with the first cysteine from the amino terminus in SEQ ID NO:2.

The encoded polypeptide has two observed leader sequences of 24 and 28 amino acids; and the amino acid sequence of the observed mature CKβ-13 proteins are also shown in Figure 1 (SEQ ID NO:2), as amino acid residues 25-93 and 29-93.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the observed mature CKβ-13 polypeptide having the amino acid sequence at positions 25-93 in SEQ ID NO:2; (c) a nucleotide sequence encoding the observed mature CKβ-

13 polypeptide having the amino acid sequence at positions 29-93 in SEQ ID NO:2; (d) a nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113; (e) a nucleotide sequence encoding a mature CKβ-13 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e) above.

An additional embodiment of this aspect of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a CKβ-13 polypeptide having an amino acid sequence described in (a), (b), (c), (d) or (e), above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a CKβ-13 polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

In another embodiment, the invention provides an isolated antibody that binds specifically to a CKβ-13 polypeptide having an amino acid sequence described in (a), (b), (c), (d) or (e) above. The invention further provides methods for isolating antibodies that bind specifically to a CKβ-13 polypeptide having an amino acid sequence as described herein. Such antibodies are useful diagnostically or therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising CKβ-13 polypeptides, particularly human CKβ-13 polypeptides, which may be employed, for instance, to treat solid tumors, chronic infections, leukemia, T-cell mediated auto-immune diseases, parasistic infections, psoriasis, to regulate hematopoiesis, to stimulate growth factor activity, to treat fibrotic disorders, to

inhibit angiogenesis and to promote wound healing. CKβ-13 may also be employed to treat sepsis and is useful for immune enhancement or suppression, myeloprotection, and acute and chronic inflammatory control.

Methods of treating individuals in need CKβ-13 polypeptides are also provided.

The invention further provides compositions comprising a CKβ-13 polynucleotide or a CKβ-13 polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a CKβ-13 polynucleotide for expression of a CKβ-13 polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a CKβ-13.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on CKβ-13 binding to a CKβ-13 receptor. In particular, the method involves contacting the CKβ-13 receptor with a CKβ-13 polypeptide and a candidate compound and determining whether CKβ-13 polypeptide binding to the CKβ-13 is increased or decreased due to the presence of the candidate compound. In this assay, an increase in binding of CKβ-13 over the standard binding indicates that the candidate compound is an agonist of CKβ-13 binding activity and a decrease in CKβ-13 binding compared to the standard indicates that the compound is an antagonist of CKβ-13 binding activity.

It has been discovered that CKβ-13 is expressed not only in monocytes but also in activated dendritic cells. For a number of disorders of theses tissues or cells, particularly of the immune system, significantly higher or lower levels of CKβ-13 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid)

taken from an individual having such a disorder, relative to a "standard" CKβ-13 gene expression level, i.e., the CKβ-13 expression level in healthy tissue from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of such a disorder, which involves: (a) assaying CKβ-13 gene expression level in cells or body fluid of an individual; (b) comparing the CKβ-13 gene expression level with a standard CKβ-13 gene expression level, whereby an increase or decrease in the assayed CKβ-13 gene expression level compared to the standard expression level is indicative of disorder in the immune.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of CKβ-13 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated CKβ-13 polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of CKβ-13 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an CKβ-13 antagonist. Preferred antagonists for use in the present invention are CKβ-13-specific antibodies.

Brief Description of the Figures

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of CKβ-13.

Figure 2 shows the regions of identity between the amino acid sequences of the CKβ-13 protein and translation product of the human mRNA for monocyte chemotactic protein-1α (MIP-1α) (lower line) (SEQ ID NO:3), determined by the computer program Bestfit (Wisconsin Sequence Analysis Package, Version 8 for Unix,

Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) using the default parameters.

Figure 3 shows an analysis of the CKβ-13 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, the indicate location of the highly antigenic regions of the CKβ-13 protein, i.e., regions from which epitope-bearing peptides of the invention may be obtained.

Figure 4 shows the chemotactic activity of CKβ-13 on activated T-lymphocytes taken from 3 donors as described in Example 5.

Detailed Description

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a CKβ-13 polypeptide having the amino acid sequence shown in SEQ ID NO:2, which was determined by sequencing a cloned cDNA. The nucleotide sequence shown in Figure 1 (SEQ ID NO:1) was obtained by sequencing the HMSDB49 clone, which was deposited on April 12, 1995 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given accession number ATCC 97113. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

The polypeptide of the present invention has amino acid sequence homology to known chemokines, including the conserved cysteine pattern characteristic of the beta subfamily of chemokines beginning with the first cysteine from the amino terminus in SEQ ID NO:2.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as the nucleotide sequence in Figure 1 (SEQ ID NO:1), a nucleic acid molecule of the present invention encoding a CKβ-13 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material.

Illustrative of the invention, the nucleic acid molecule described in Figure 1 (SEQ ID NO:1) was discovered in a cDNA library derived from human monocytes.

Additional clones of the same gene were also identified in cDNA libraries from activated dendritic cells.

The determined nucleotide sequence of the CKβ-13 cDNA of Figure 1 (SEQ ID NO:1) contains an open reading frame encoding a protein of 93 amino acid residues, with an initiation codon at nucleotide positions 1-3 of the nucleotide sequence in Figure 1 (SEQ ID NO:1). The amino acid sequence of the CKβ-13 protein shown in SEQ ID NO:2 is about 33% identical to and 53% similar to human mRNA for MIP-1α (Figure 2).

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual complete CKβ-13 polypeptide encoded by the deposited cDNA, which comprises about 93 amino acids, may be somewhat longer or shorter. More generally, the actual open reading frame may be anywhere in the range of ±20 amino acids, more likely in the range of ±10 amino acids, of that predicted from the first methionine codon from the N-terminus shown in Figure 1 (SEQ ID NO:1).

Leader and Mature Sequences

The amino acid sequence of the complete CKβ-13 protein is shown in SEQ ID NO:2 and includes leader sequences and mature protein(s), as described below. More in particular, the present invention provides nucleic acid molecules encoding a mature form of the CKβ-13 protein. Thus, according to the signal hypothesis, once export of the growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the complete polypeptide to produce a secreted "mature" form of the

protein. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature CKβ-13 polypeptide having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 97113. By the "mature CKβ-13 polypeptide having the amino acid sequence encoded by the cDNA clone in ATCC Deposit No. 97113" is meant the mature form(s) of the CKβ-13 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host.

In the present case, the deposited cDNA has been expressed in a baculovirus vector in insect cells as described herein below, and amino acid sequencing of the amino terminus of the two secreted species indicated that the mature CKβ-13 proteins comprise amino acids 25 to 93 and 29 to 93 of SEQ ID NO:2. Thus, the leader sequences of the CKβ-13 protein in the amino acid sequence of SEQ ID NO:2 are 24 and 28 amino acids, respectively.

In addition, methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res. 3*:271-286 (1985)) uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2 where +1 indicates the amino terminus of the mature protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80% (von Heinje, *supra*). However, the

two methods do not always produce the same predicted cleavage point(s) for a given protein.

As one of ordinary skill would appreciate from the above discussions, due to the possibilities of sequencing errors as well as the variability of cleavage sites in different known proteins, the twp mature CKβ-13 polypeptide species encoded by the deposited cDNA are expected to consist of about 65 and 69 amino acids, but may consist of any number of amino acids in the range of about 58-73 amino acids; and the actual leader sequences of this protein are expected to be 24 and 28 amino acids, but may consist of any number of amino acids in the range of 20-35 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 1-3 of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1).

Also included are DNA molecules comprising the coding sequence for the observed mature CKβ-13 protein.

In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the CKβ-13 protein. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

In another aspect, the invention provides isolated nucleic acid molecules encoding the CKβ-13 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97113 on April 28, 1995. Preferably, this nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited cDNA clone.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the CKβ-13 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the CKβ-13 gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein as well as to fragments of the isolated nucleic acid molecules described herein. In particular, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 1-279.

More generally, by a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-300 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in Figure 1 (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1). Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the CKβ-13 polypeptide as identified in Figure 3 and described in more detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clone contained in ATCC Deposit No. 97113. By "stringent hybridization conditions" is intended overnight incubation at 42° C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65° C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the CKβ-13 cDNA shown in Figure 1 (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a CKβ-13 polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptide, by itself; and the coding sequence for the mature polypeptide and additional sequences, such as those encoding the about 20-35 amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences.

Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in

a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*: 767 (1984). As discussed below, other such fusion proteins include the CKβ-13 fused to Fc at the N- or C-terminus.

Variant and Mutant Polynucleotides

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the CKβ-13 protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the CKβ-13 protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Most highly preferred are nucleic acid molecules encoding the mature protein having the amino acid sequence described above or the mature CKβ-13 amino acid sequence encoded by the deposited cDNA clone.

Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the observed mature CKβ-13 polypeptide having the amino acid sequence at positions 25 to 93 of SEQUENCE ID NO:2; (c) a nucleotide sequence encoding the observed mature CKβ-13 polypeptide having the amino acid sequence at positions 29 to 93 of SEQUENCE ID NO:2; (d) a nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113; (e) a nucleotide sequence encoding a mature CKβ-13 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e) above.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f), above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e) or (f), above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a CKβ-13 polypeptide having an amino acid sequence in (a), (b), (c), (d) or (e), above.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of CKβ-13 polypeptides or peptides by recombinant techniques.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a CKβ-13 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the CKβ-13 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotides sequence of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to

determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having CKβ-13 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having CKβ-13 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having CKβ-13 activity include, inter alia, (1) isolating the CKβ-13 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the CKβ-13 gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press. New York (1988); and Northern Blot analysis for detecting CKβ-13 mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having CKβ-13 protein activity. By "a polypeptide having CKβ-13 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the mature protein of the invention, as measured in a particular biological assay. For example, the CKβ-13 protein of the present invention is chemotactic for activated T-lymphocytes in the assay described in Example 5.

CKβ-13 protein is chemotactic in a dose-dependent manner for activated T-lymphocytes in the above-described assay. Thus, "a polypeptide having CKβ-13 protein activity" includes polypeptides that also exhibit any of the same activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the CKβ-13 protein, preferably, "a polypeptide having CKβ-13 protein activity" will exhibit substantially similar dose-dependence in a given activity as compared to the CKβ-13 protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference CKβ-13 protein).

Like other CC chemokines, CKβ-13 exhibits activity on leukocytes with a strong activity on T-lymphocytes which have been activiated by cross-linking of the CD3 receptor in the presence of IL-2. For this reason CKβ-13 is active in directing the proliferation, differentiation and migration of these cell types. Such activity is useful for immune enhancement or suppression, myeloprotection, stem cell mobilization, acute and chronic inflammatory control and treatment of leukemia. However, unlike other known CC chemokines CKβ-13 has been shown to be expressed only in an activated monocyte and dendritic cell cDNA library. These two cell types combined make up the majority of the anitigen presenting cells (APCs). Dendritic cells (DCs) and monocytes are professional APCs which are critical for the proper response of the host and are responsible for primary antigen-specific immune reactions. APCs play a crucial role in the presentation of antigens to both T-lymphocytes and B-lymphocytes to initiate the immune response, including for example, antigen trapping and processing, viral trapping, filtering and processing. APCs are normally found in the lymph node, spleen, thymus, skin and circulate throughout the body. When found in the skin, DCs are refered to as Langerhans cells. Follicular dendritic cells reside in the germinal centers of the lymph node. Because

CKβ-13 is produced by these cells, CKβ-13 is active in modulating the activities of both monocytes and dendritic cells as well as the cells with which these APCs interact. In addition, CKβ-13 has effects on the local resident cells in which APCs normally reside such as the skin, thymus, spleen, and lymph node.

CKβ-13 regulates the proliferation and maturation of DCs and is monitored in a proliferation/differentiation assay such as those reviewed by Peters *et al.* (1996) *Immun. Today* 17:273 and described by Young *et al.* (1995) *J. Exp. Med.* 182:1111; Caux *et al.* (1992) *Nature* 360:258; and Santigo-Schwarz *et al.* (1995) *Adv. Exp. Med. Biol.* 378:7. Representative cell lines could also be employed in such assays. CKβ-13 also influences the effector function of DCs and monocytes. That is, CKβ-13 enhaces the capacity of DCs and monocytes to take up virus, bacteria or other foreign substances, process them and present them to the lymphocytes responsible for immune responses. CKβ-13 also modulates the interaction of DCs and monocytes with T-lymphocytes and B-lymphocytes. For instance, CKβ-13 provides a costimulation signal during antigen presentation which directs the responding cell to survive, proliferate, differentiate, secrete additional cytokines or soluble mediators, or selectively removes the responding cell by inducing apoptosis or other mechanisms of cell death. Since DCs and monocytes have been shown to facilitate the transfer of HIV to CD4+ T-lymphocytes CKβ-13 also influences this ability and prevents infection of lymphocytes by HIV or other viruses mediated through monocytes or DCs. This is also true for the intital infection of monocytes and DCs by such viruses.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) will encode a polypeptide "having CKβ-13 protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same

polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having CKβ-13 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of CKβ-13 polypeptides or fragments thereof by recombinant techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating

codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, Streptomyces and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., *supra*; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to

facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al., J. Molecular Recognition 8*:52-58 (1995) and K. Johanson *et al., J. Biol. Chem. 270*:9459-9471 (1995).

The CKβ-13 protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Polypeptides and Fragments

The invention further provides an isolated CKβ-13 polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NO:2, or a peptide or polypeptide comprising a portion of the above polypeptides.

Variant and Mutant Polypeptides

To improve or alter the characteristics of CKβ-13 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the

corresponding natural polypeptide, at least under certain purification and storage conditions.

N-Terminal and C-Terminal Deletion Mutants

For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., *J. Biol. Chem., 268*:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. In the present case, since the protein of the invention is a member of the chemokine polypeptide family, deletions of N-terminal amino acids up to the Cys at position 36 of SEQ ID NO:2 may retain some biological activity such as receptor binding or modulation of target cell activities, for chemokines. Polypeptides having further N-terminal deletions including the Cys-36 residue in SEQ ID NO:2 would not be expected to retain such biological activities because it is known that this residue in a chemokine-related polypeptide is required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the CKβ-13 shown in SEQ ID NO:2, up to the Cys-36 residue, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n-93 of SEQ ID NO:2, where n is an integer in the range of 1-35 where Cys-36 is the position of the first residue from the N-terminus of the complete CKβ-13 polypeptide (shown in SEQ ID NO:2) believed to be required for receptor binding activity of the CKβ-13 protein.

More in particular, the invention provides polypeptides having the amino acid sequence of residues 1-93, 2-93, 3-93, 4-93, 5-93, 6-93, 7-93, 8-93, 9-93, 10-93, 11-93, 12-93, 13-93, 14-93, 15-93, 16-93, 17-93, 18-93, 19-93, 20-93, 21-93, 22-93, 23-93, 24-93, 25-93, 26-93, 27-93, 28-93, 29-93, 30-93, 31-93, 32-93, 33-93, 34-93, and 35-93 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988). In the present case, since the protein of the invention is a member of the chemokine polypeptide family, deletions of C-terminal amino acids up to the Cys at position 76 of SEQ ID NO:2 may retain some biological activity such as receptor binding or modulation of target cell activities, for chemokines. Polypeptides having further C-terminal deletions including Cys-76 of SEQ ID NO:2 would not be expected to retain such biological activities because it is known that this residue in a chemokine-related polypeptide is required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the

protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the CKβ-13 shown in SEQ ID NO:2, up to the Cys-76 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m of the amino acid sequence in SEQ ID NO:2, where m is any integer in the range of 77 to 93 where 76 is the position of the C- terminal Cys residue of the complete CKβ-13 polypeptide (shown in SEQ ID NO:2) believed to be required for receptor binding or modulation of target cell activities of the CKβ-13 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues n-m, where n is an integer 1-35 and m is an integer 77-93 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete CKβ-13 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113, where this portion excludes from 1 to about 35 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113, or from 1 to about 17 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded

by the cDNA clone contained in ATCC Deposit No. 97113. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

Other Mutants

In addition to terminal deletion forms of the protein discussed above, it also will be recognized by one of ordinary skill in the art that some amino acid sequences of the CKβ-13 polypeptide can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

Thus, the invention further includes variations of the CKβ-13 polypeptide which show substantial CKβ-13 polypeptide activity or which include regions of CKβ-13 protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent

substitutions are described in Bowie, J. U. *et al., supra*, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2, or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the CKβ-13 of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the CKβ-13 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* or *in vitro* proliferative activity.

Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al., Clin. Exp. Immunol. 2*:331-340 (1967); Robbins

et al., Diabetes 36: 838-845 (1987); Cleland *et al., Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993).

Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the CKβ-13 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using anti-CKβ-13 antibodies of the invention in methods which are well known in the art of protein purification.

Further polypeptides of the present invention include polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. The polypeptides of the invention also comprise those which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA or to the polypeptide of SEQ ID NO:2, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711)

and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a CKβ-13 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the CKβ-13 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2 or to the amino acid sequence encoded by deposited cDNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that

gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting CKβ-13 protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting CKβ-13 protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" CKβ-13 protein binding proteins which are also candidate agonists and antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, Nature 340:245-246 (1989).

Epitope-Bearing Portions

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green,

N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins," *Science, 219*:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate CKβ-13-specific antibodies include: a polypeptide comprising amino acid residues from about Thr-22 to about Gly-28; Asn-30 to about Leu-47; Thr-56 to about Val-65; and Phe-70 to about Trp-83. These polypeptide fragments have been determined to bear antigenic epitopes of the CKβ-13 protein by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 3, above.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids." *Proc. Natl. Acad. Sci. USA 82*:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et

al., supra; Wilson et al., supra; Chow, M. *et al., Proc. Natl. Acad. Sci. USA* *82*:910-914; and Bittle, F. J. *et al., J. Gen. Virol. 66*:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Fusion Proteins

As one of skill in the art will appreciate, CKβ-13 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Fusion proteins that have a

disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric CKβ-13 protein or protein fragment alone (Fountoulakis et *al., J. Biochem. 270*:3958-3964 (1995)).

Antibodies

CKβ-13-protein specific antibodies for use in the present invention can be raised against the intact CKβ-13 protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to CKβ-13 protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the CKβ-13 protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of CKβ-13 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or CKβ-13 protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Köhler *et al., Nature 256*:495 (1975); Köhler *et al., Eur. J. Immunol. 6*:511 (1976); Köhler et al.,

Eur. J. Immunol. 6:292 (1976); Hammerling *et al.*, *in*: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a CKβ-13 protein antigen or, more preferably, with a CKβ-13 protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-CKβ-13 protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the CKβ-13 protein antigen.

Alternatively, additional antibodies capable of binding to the CKβ-13 protein antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, CKβ-13-protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the CKβ-13 protein-specific antibody can be blocked by the CKβ-13 protein antigen. Such antibodies comprise anti-idiotypic antibodies to the CKβ-13 protein-specific

antibody and can be used to immunize an animal to induce formation of further CKβ-13 protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, CKβ-13 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of anti-CKβ-13 in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al., Nature 314*:268 (1985).

Immune System-Related Disorders

Diagnosis

The present inventors have discovered that CKβ-13 is expressed inactivated monocytes and ex vivo expanded dendritic cells. For a number of immune system-related disorders, substantially altered (increased or decreased) levels of CKβ-13 gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" CKβ-13 gene expression level, that is, the CKβ-13 expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, which involves measuring the expression level of the gene encoding the CKβ-13 protein in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard CKβ-13 gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

In particular, it is believed that certain tissues in mammals with cancer of the immune system express significantly altered (i.e., either enhanced or decreased) levels of the CKβ-13 protein and mRNA encoding the CKβ-13 protein when compared to a corresponding "standard" level. Further, it is believed that altered levels of the CKβ-13 protein can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, including cancers of this system which involves measuring the expression level of the gene encoding the CKβ-13 protein in immune system tissue or other cells or body fluid from an individual and comparing the measured gene

expression level with a standard CKβ-13 gene expression level, whereby a significant increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

Where a diagnosis of a disorder in the immune system, including diagnosis of a tumor has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting a significantly altered CKβ-13 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "assaying the expression level of the gene encoding the CKβ-13 protein" is intended qualitatively or quantitatively measuring or estimating the level of the CKβ-13 protein or the level of the mRNA encoding the CKβ-13 protein in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the CKβ-13 protein level or mRNA level in a second biological sample). Preferably, the CKβ-13 protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard CKβ-13 protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard CKβ-13 protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains CKβ-13 protein or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free CKβ-13 protein, immune system tissue, and other tissue sources found to express complete or mature CKβ-13 or a CKβ-13 receptor. Methods for obtaining tissue biopsies and body

fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The present invention is useful for diagnosis or treatment of various immune system-related disorders, including disregulation of immune cell function in mammals, preferably humans. Such disorders include tumors, cancers, interstitial lung disease (such as Langerhans cell granulomatosis) and any disregulation of immune cell function including but not limited to, leukemias, lymphomas, autoimmune diseases, arthritis, immune suppression, histamine and IgE-mediated allergic reactions, sepsis, prostaglandin-independant fever, bone marrow failure, wound healing, silicosis, sarcoidosis, acute and chronic infection, cell mediated immunity, humoral immunity, inflammatory bowel disease, mylosuppression and hyper-eosinophil syndrome and the like.

Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the CKβ-13 protein are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying CKβ-13 protein levels in a biological sample can occur using antibody-based techniques. For example CKβ-13 protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)). Other antibody-based methods useful for detecting CKβ-13 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I,

^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying CKβ-13 protein levels in a biological sample obtained from an individual, CKβ-13 protein can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of CKβ-13 protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A CKβ-13 protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain CKβ-13 protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Treatment

As noted above, CKβ-13 polynucleotides and polypeptides are useful for diagnosis of conditions involving abnormally high or low expression of CKβ-13

activities. Given the cells and tissues where CKβ-13 is expressed as well as the activities modulated by CKβ-13, it is readily apparent that a substantially altered (increased or decreased) level of expression of CKβ-13 in an individual compared to the standard or "normal" level produces pathological conditions related to the bodily system(s) in which CKβ-13 is expressed and/or is active.

It will also be appreciated by one of ordinary skill that, since the CKβ-13 protein of the invention is a member of the chemokine beta family the mature form(s) of the protein may be released in soluble form from the cells which express the CKβ-13 by proteolytic cleavage. Therefore, when mature CKβ-13 is added from an exogenous source to cells, tissues or the body of an individual, the protein will exert its physiological activities on its target cells of that individual.

Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of CKβ-13 activity in an individual, particularly disorders of the immune system, can be treated by administration of CKβ-13 polypeptide (in the form of mature protein. Thus, the invention also provides a method of treatment of an individual in need of an increased level of CKβ-13 activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated CKβ-13 polypeptide of the invention, particularly a mature form of the CKβ-13 effective to increase the CKβ-13 activity level in such an individual.

The polypeptides of the present invention may be emplyed to inhibit bone marrow stem cell colony formation as an adjunct protective treatment during cancer chemotherapy. The CKβ-13 polypeptide may inhibit the proliferation and differentiation of hematopoietic cells such as bone marrow stem cells. The inhibitor effect on the population of committed progenitor cells, (for example, granulocytes, and macrophages/monocytes) may be employed therapeutically to inhibit proliferation of leukemic cells.

The polypeptides of the present invention may also be employed to inhibit epidermal keratinocyte proliferation for treatment of psoriasis, which is characterized by keratinocyte hyper-proliferation, since Langerhans cells in skin have been found to produce chemokines.

CKβ-13 may be employed as an anti-neovascularizing agent to treat solid tumors; e.g., Karposi sarcoma by stimulating the invasion and activation of host defense cells; e.g., cytotoxic T cells and macrophages and by inhibiting the angiogenesis of tumors. Those of skill in the art will recognize other non-cancer indications where blood vessel proliferation is not wanted.

CKβ-13 polypeptides may be employed to enhance host defenses against resistant chronic and acute infections, for example, mycobacterial infections via the attraction and activation of microbicidal leukocytes.

CKβ-13 may also be employed to inhibit T-cell proliferation by the inhibition of IL-2 biosynthesis for the treatment of T-cell mediated auto-immune diseases and lymphocytic leukemias.

CKβ-13 may also be employed to stimulate wound healing and prevent scarring during healing, both via the recruitment of debris clearing and connective tissue promoting inflammatory cells and also via its control of excessive TGF - mediated fibrosis. In this same manner, CKβ-13 may also be employed to treat other fibrotic disorders, including liver cirrhosis, osteoarthritis and pulmonary fibrosis.

CKβ-13 also increases the presence of eosinophils which have the distinctive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis. CKβ-13 also increases the presence of and activates Natural Killer (NK) cells which will be useful for treating a variety of diseases in which the presence of NK cells are beneficial well known to those of skill in the art.

It may also be employed to regulate hematopoiesis, by regulating the activation and differentiation of various hematopoietic progenitor cells, for example, to

release mature leukocytes from the bone marrow following chemotherapy, i.e., in stem cell mobilization.

CKβ-13 may also be employed to treat sepsis and is useful for immune enhancement or suppression, myeloprotection, and acute and chronic inflammatory control.

They may also be employed to regulate hematopoiesis, by regulating activation and differentiation of various hematopoietic progenitor cells, for example, to release mature leukocytes from the bone marrow following chemotherapy.

The polypeptides of the present invention may also be used to target unwanted cells, such as in the treatment of cancer, for apoptosis.

The polypeptide may also be used to mobilize bone marrow stem cells to peripheral blood, which allows easy isolation of stem cells. The isolation of stem cells may be employed for bone marrow colonization after high dose chemotherapy.

Formulations

The CKβ-13 polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with CKβ-13 polypeptide alone), the site of delivery of the CKβ-13 polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of CKβ-13 polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of CKβ-13 polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the CKβ-13 polypeptide is typically

administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the CKβ-13 of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The CKβ-13 polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers 22*:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release CKβ-13 polypeptide compositions also include liposomally entrapped CKβ-13 polypeptide. Liposomes containing CKβ-13 polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800

Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal CKβ-13 polypeptide therapy.

For parenteral administration, in one embodiment, the CKβ-13 polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the CKβ-13 polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol

or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The CKβ-13 polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of CKβ-13 polypeptide salts.

CKβ-13 polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic CKβ-13 polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

CKβ-13 polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous CKβ-13 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized CKβ-13 polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Agonists and Antagonists - Assays and Molecules

The invention also provides a method of screening compounds to identify those which enhance or block the action of CKβ-13 on cells, such as its interaction with CKβ-13-binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of CKβ-13 or which functions in a manner similar to CKβ-13, while antagonists decrease or eliminate such functions.

In another aspect of this embodiment the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a CKβ-13 polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds CKβ-13. The preparation is incubated with labeled CKβ-13 and complexes of CKβ-13 bound to the receptor or other binding protein are isolated and characterized according to routine methods known in the art. Alternatively, the CKβ-13 polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

In the assay of the invention for agonists or antagonists, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds CKβ-13, such as a molecule of a signaling or regulatory pathway modulated by CKβ-13. The preparation is incubated with labeled CKβ-13 in the absence or the presence of a candidate molecule which may be a CKβ-13 agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of CKβ-13 on binding the CKβ-13 binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to CKβ-13 are agonists.

CKβ-13-like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of CKβ-13 or molecules that elicit the same effects as CKβ-13. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for CKβ-13 antagonists is a competitive assay that combines CKβ-13 and a potential antagonist with membrane-bound CKβ-13 receptor molecules or recombinant CKβ-13 receptor molecules under appropriate conditions for a competitive inhibition assay. CKβ-13 can be labeled, such as by radioactivity, such that the number of CKβ-13 molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing CKβ-13-induced activities, thereby preventing the action of CKβ-13 by excluding CKβ-13 from binding.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem. 56*: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression." CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance *Lee et al., Nucleic Acids Research 6*: 3073 (1979); Cooney *et al.,*

Science 241: 456 (1988); and *Dervan et al., Science 251*: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of CKβ-13. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into CKβ-13 polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of CKβ-13 protein.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

The antagonists may be employed for instance to inhibit the chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of auto-immune diseases include multiple sclerosis, and insulin-dependent diabetes.

The antagonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the human chemokine polypeptides of the present invention.

The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall.

The antagonists may also be employed to treat histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated.

The antagonists may also be employed to treat chronic and acute inflammation by preventing the attraction of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung.

Antagonists may also be employed to treat rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients. Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies.

The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory cytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by chemokines.

The antagonists may also be employed to treat cases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome.

The antagonists may also be employed to treat asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung.

Antibodies against CKβ-13 may be employed to bind to and inhibit CKβ-13 activity to treat, for example, ARDS, by preventing infiltration of neutrophils into the lung after injury.

Any of the above antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a CKβ-13 protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1(a): Expression and Purification of CKβ-13 in E. coli

The bacterial expression vector pQE60 was used for bacterial expression in this example (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide

coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

The DNA sequence encoding the desired portion of the CKβ-13 protein comprising the mature form beginning with Gly-25 of the CKβ-13 amino acid sequence was amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the CKβ-13 protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector were added to the 5' and 3' sequences, respectively.

For cloning the mature form of the CKβ-13 protein beginning with Gly-25, the 5' primer has the sequence 5' AAACCATGGGTCCGTACGGTGCAAACATGGAAGACAGCG 3' (SEQ ID NO:4) containing the underlined NcoI restriction site (bold). Particular nucleotides in the "wobble" position in certain codons in both primers have been altered based on E. coli preference. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the mature form. The 3' primer has the sequence 5' AAAAAGCTTCTGACCCTTCCCTGGAAGGTA 3' (SEQ ID NO:5) containing the underlined HindIII restriction site.

The amplified CKβ-13 DNA fragments and the vector pQE60 were digested with NcoI and HindIII and the digested DNAs were then ligated together. Insertion of the CKβ-13 DNA into the restricted pQE60 vector places the CKβ-13 protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture was transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing CKβ-13 protein, is available commercially from QIAGEN, Inc., *supra*. Transformants were identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA was isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs were grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture was used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells were grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. isopropyl-b-D-thiogalactopyranoside ("IPTG") was then added to a final concentration of 1 mM to induce transcription from the *lac* repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently were incubated further for 3 to 4 hours. Cells then were harvested by centrifugation.

To purify the CKβ-13 polypeptide, the cells were then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris was removed by centrifugation, and the supernatant containing the CKβ-13 was dialyzed against 50 mM Na-acetate buffer pH 6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure CKβ-13 protein. The purified protein is stored at 4° C or frozen at -80° C.

The following alternative method may be used to purify CKβ-13 expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells are harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells ware then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the

pellet is discarded and the CKβ -13 polypeptide-containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded CKβ-13 polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 mm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the CKβ-13 polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the CKβ-13 polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant CKβ-13 polypeptide exhibits greater than 95% purity after the above refolding and purification steps. No major contaminant bands are observed

from Commassie blue stained 16% SDS-PAGE gel when 5 μg of purified protein is loaded. The purified protein is also tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 2: Cloning and Expression of CKβ-13 protein in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 was used to insert the cloned DNA encoding complete protein, including its naturally associated secretory signal (leader) sequence, into a baculovirus to express the mature CKβ-13 protein, using standard methods as described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology 170*:31-39 (1989).

The cDNA sequence encoding the full length CKβ-13 protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:2, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' AAA**GGATCC**GCCACCATGGCTCGCCTACAGACT 3' (SEQ ID NO:6) containing a BamHI restriction enzyme site (bold), and an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987). The 3' primer has the sequence 5' AAA**GGTACC**TCATTGGCTCAGCTTATT 3' (SEQ ID NO:7) containing an Asp718 restriction enzyme site (bold).

The amplified fragment was isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then was digested with BamHI and Asp718 and again is purified on a 1% agarose gel.

The plasmid was digested with the restriction enzymes BamHI and Asp718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA was then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and dephosphorylated plasmid were ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells were transformed with the ligation mixture and spread on culture plates. Bacteria were identified that contain the plasmid with the human CKβ-13 gene by digesting DNA from individual colonies using BamHI and Asp718 and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment was confirmed by DNA sequencing. This plasmid is designated herein pA2CKβ-13.

Five μg of the plasmid pA2CKβ-13 was co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus

DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA 84*: 7413-7417 (1987). One μg of BaculoGold™ virus DNA and 5 μg of the plasmid pA2CKβ-13 were mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture was added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate was then incubated for 5 hours at 27° C. The transfection solution was then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum was added. Cultivation was then continued at 27° C for four days.

After four days the supernatant was collected and a plaque assay was performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) was used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques were picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses was then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus was used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes were harvested and then stored at 4° C. The recombinant virus is called V-CKβ-13.

To verify the expression of the CKβ-13 gene Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with

the recombinant baculovirus V- CKβ-13 at a multiplicity of infection ("MOI") of about 2. 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) were added. The cells were further incubated for 16 hours and then harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins were analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified proteins was used to determine the amino terminal sequence of the mature of the CKβ-13 protein, and thus the leader and mature forms, as described above.

Example 3: Expression of Recombinant CKβ-13 in COS Cells

The expression of plasmid CKβ-13HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire CKβ-13 precursor and an HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemaglutinin protein as previously described (I. Wilson et al., 1984, Cell 37, 767). The fusion of an HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding CKβ-13, ATCC # 97113, is constructed by PCR using two primers: The 5' primer

5' AAAAAGCTTAACATAGGCTCGCCTACAGACT 3' (SEQ ID NO:8) contains a HindIII site followed by 18 nucleotides of CKβ-13 coding sequence starting from the minus 3 position relative to the initiation codon; the 3' primer 5'CGCTCTAGATTAAGCGTAGTCTGGGACGTCGTATGGGTATTGGCTCA GCTTATTGAGAAT 3' (SEQ ID NO:9) contains complementary sequence to an XbaI site, translation stop codon, HA tag and the last 21 nucleotides of the CKβ-13 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, CKβ-13 coding sequence followed by an HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XbaI site. The PCR amplified DNA fragment and the vector, pcDNA3/Amp, are digested with HindIII and XbaI restriction enzyme and ligated. The ligation mixture is transformed into E. coli strain SURE (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant CKβ-13 polypeptide, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1989)). The expression of the CKβ-13HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with 35-S-Cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media are precipitated with an HA specific monoclonal antibody. Proteins precipitated are analyzed by SDS-PAGE.

Example 4: Expression via Gene Therapy

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temp. over night. After 24 hours at room temp., the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media, e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin, is added. This is then incubated at 37 degrees C for approximately one week. At this time fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al. DNA, 7:219-25 (1988) flanked by the long terminal repeats of the Moloney murine sarcoma virus is digested with EcoRI and HindIII and subsequently treated with calf intestinal alkaline phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention is amplified using PCR primers which correspond to the 5' and 3' end sequences respectively. The 5' primer containing an EcoRI site and the 3' primer further includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is used to transform bacteria HB101, which are then plated onto agar-containing kanamycin for the purpose of confirming that the vector had the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with

10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells are transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently the media is harvested from a 10cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titier is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product.

Example 5: Chemotactic Effect of CKβ-13 on Activated T-lymphocytes

Peripheral blood mononuclear cells were purified from donor leukopacks (Red Cross) by centrifugation on lymphocyte separation medium (LSM; density 1.077 g/ml; Organon Teknika Corp.) and harvesting the interface band. T-lymphocytes purified from the PBMCs using T-cell enrichment columns (R&D Systems). For activation of the T-lymphocytes, cells were stimulated by crosslinking through the CD3 receptor in the presence of IL-2 (10 U/ml) for 16 hours prior to the chemotaxis assay. Cells used for the assay were washed 3X with HBSS/0.1% BSA and resuspended @ 2 x 106/ml for labeling. Calcein-AM (Molecular Probes) was added to a final concentration of 1 mM and the cells incubated at 37°C for 30 minutes. Following this incubation the cells were washed 3X with HBSS/0.1% BSA. Labeled

cells were resuspended as 4-8 x 106/ml and 25ml (1-2 x 105 cells) added to the top of a polycarbonate filter (3-5 mm pore size; PVP free; NeuroProbe, Inc.) which separates the cell suspension from the chemotactic agent in the plate below. Cells are allowed to migrate for 45 - 90 minutes and then the number of migrated cells (both attached to the filter as well as in the bottom plate) are quantitated using a Cytofluor II fluorescence plate reader (PerSeptive Biosystems).

Activated T-lymphocytes from three different donors were used for chemotaxis assays as described above. The data for MCP-1 (open circles) and CkBeta-13 (closed triangles) are presented as the chemotactic index (the ratio between the number of cells migrated in the presence of chemokines and the number of cells migrated in the presence of buffer control) in Figure 4.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LI, HAODONG
SEIBUL, GEORGE

(ii) TITLE OF INVENTION: HUMAN CHEMOKINE BETA 13

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: 20850
(F) ZIP: US

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: PF177PCT2

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 282 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..279

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG GCT CGC CTA CAG ACT GCA CTC CTG GTT GTC CTC GTC CTC CTT GCT        48
Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala
 1               5                  10                  15

GTG GCG CTT CAA GCA ACT GAG GCA GGC CCC TAC GGC GCC AAC ATG GAA        96
Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr Gly Ala Asn Met Glu
            20                  25                  30

GAC AGC GTC TGC TGC CGT GAT TAC GTC CGT CAC CGT CTG CCC CTG CGC       144
Asp Ser Val Cys Cys Arg Asp Tyr Val Arg His Arg Leu Pro Leu Arg
        35                  40                  45

GTG GTG AAA CAC TTC TAC TGG ACC TCA GAC TCC TGC CCG AGG CCT GGC       192
Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly
    50                  55                  60

GTG GTG TTG CTA ACC TTC AGG GAT AAG GAG ATC TGT GCC GAT CCC AGA       240
Val Val Leu Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro Arg
65                  70                  75                  80

GTG CCC TGG GTG AAG ATG ATT CTC AAT AAG CTG AGC CAA TGA               282
Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu Ser Gln
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala
 1               5                  10                  15

Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr Gly Ala Asn Met Glu
            20                  25                  30

Asp Ser Val Cys Cys Arg Asp Tyr Val Arg His Arg Leu Pro Leu Arg
        35                  40                  45

Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly
    50                  55                  60

Val Val Leu Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro Arg
65                  70                  75                  80

Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu Ser Gln
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
1 5 10 15

Leu Cys Asn Gln Phe Ser Ala Ser Leu Ala Ala Asp Thr Pro Thr Ala
20 25 30

Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile Ala
35 40 45

Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Gly Val Ile Phe
50 55 60

Leu Thr Lys Arg Ser Arg Gln Val Cys Ala Asp Pro Ser Glu Glu Trp
65 70 75 80

Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
85 90

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAACCATGGG TCCGTACGGT GCAAACATGG AAGACAGCG 39

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAAAAGCTTC TGACCCTTCC CTGGAAGGTA 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAAGGATCCG CCACCATGGC TCGCCTACAG ACT 33

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAGGTACCT CATTGGCTCA GCTTATT 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAAAAGCTTA ACATAGGCTC GCCTACAGAC T 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCTCTAGAT TAAGCGTAGT CTGGGACGTC GTATGGGTAT TGGCTCAGCT TATTGAGAAT 60

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 7, line 10

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit: April 12, 1995

Accession Number: 97113

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Form PCT/RO/134 (July 1992)

What Is Claimed Is:

1. An isolated nucleic acid molecule nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence in SEQ ID NO:2;

(b) a nucleotide sequence encoding the CKβ-13 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97113;

(c) a nucleotide sequence encoding a mature form of the CKβ-13 polypeptide having the amino acid sequence at positions 25-93 in SEQ ID NO:2;

(d) a nucleotide sequence encoding a mature form of the CKβ-13 polypeptide having the amino acid sequence at positions 29-93 in SEQ ID NO:2;

(e) a nucleotide sequence encoding a mature form of the CKβ-13 polypeptide as encoded by the cDNA clone contained in the ATCC Deposit No. 97113; and

(f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1 (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the CKβ-13 polypeptide having the complete amino acid sequence in SEQ ID NO:2.

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding a mature form of the CKβ-13 polypeptide having the amino acid sequence from about 73 to about 279 in SEQ ID NO:2.

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding a mature form of the CKβ-13 polypeptide having the amino acid sequence from about 85 to about 279 in SEQ ID NO:2.

6. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues n-93 of SEQ ID NO:2, where n is an integer in the range of 1-35;

(b) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues 1-m of SEQ ID NO:2, where m is an integer in the range of 77-93;

(c) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-m of SEQ ID NO:2, where n and m are integers as defined respectively in (a) and (b) above; and

(d) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete CKβ-13 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113 wherein said portion excludes from 1 to about 35 amino acids from the amino terminus of said complete amino acid sequence;

(e) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete CKβ-13 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113 wherein said portion excludes from 1 to about 17 amino acids from the carboxy terminus of said complete amino acid sequence; and

(f) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete CKβ-13 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113 wherein said portion include a combination of any of the amino terminal and carboxy terminal deletions in (d) and (e), above.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97113.

8. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the CKβ-13 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113.

9. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a mature CKβ-13 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97113.

10. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), or (e) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

11. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a CKβ-13 polypeptide having an amino acid sequence in (a), (b), (c), (d), or (e) of claim 1.

12. The isolated nucleic acid molecule of claim 11, which encodes an epitope-bearing portion of a CKβ-13 polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about Thr-22 to about Gly-28 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Asn-30 to about Leu-47 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Thr-56 to about Val-65 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about Phe-70 to about Trp-83 (SEQ ID NO:2).

13. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

14. A recombinant vector produced by the method of claim 13.

15. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 14 into a host cell.

16. A recombinant host cell produced by the method of claim 15.

17. A recombinant method for producing a CKβ-13 polypeptide, comprising culturing the recombinant host cell of claim 16 under conditions such that said polypeptide is expressed and recovering said polypeptide.

18. An isolated CKβ-13 polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the complete amino acid sequence in SEQ ID NO:2 or as encoded by the cDNA clone contained in ATCC Deposit No. 97113;

(b) the amino acid sequence of a mature CKβ-13 polypeptide having the amino acid sequence at positions 25-93 or 29-93 in SEQ ID NO:2, or as encoded by the cDNA clone contained in the ATCC Deposit No. 97113.

19. An isolated polypeptide comprising an epitope-bearing portion of the CKβ-13 protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about Thr-22 to about Gly-28 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Asn-30 to about Leu-47 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Thr-56 to about Val-65 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about Phe-70 to about Trp-83 (SEQ ID NO:2).

20. An isolated antibody that binds specifically to a CKβ-13 polypeptide of claim 18.

FIGURE 1

```
ATGGCTCGCCTACAGACTGCACTCCTGGTTGTCCTCGTCCTCCTTGCTGTGGCGCTTCAA
M  A  R  L  Q  T  A  L  L  V  V  L  V  L  L  A  V  A  L  Q

GCAACTGAGGCAGGCCCCTACGGCGCCAACATGGAAGACAGCGTCTGCTGCCGTGATTAC
A  T  E  A  G  P  Y  G  A  N  M  E  D  S  V  C  C  R  D  Y

GTCCGTCACCGTCTGCCCCTGCGCGTGGTGAAACACTTCTACTGGACCTCAGACTCCTGC
V  R  H  R  L  P  L  R  V  V  K  H  F  Y  W  T  S  D  S  C

CCGAGGCCTGGCGTGGTGTTGCTAACCTTCAGGGATAAGGAGATCTGTGCCGATCCCAGA
P  R  P  G  V  V  L  L  T  F  R  D  K  E  I  C  A  D  P  R

GTGCCCTGGGTGAAGATGATTCTCAATAAGCTGAGCCAATGA
V  P  W  V  K  M  I  L  N  K  L  S  Q  *
```

FIGURE 2

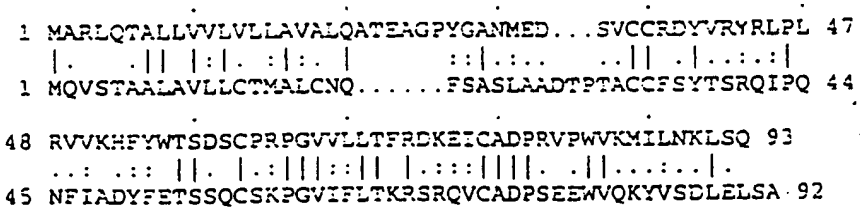

1 MARLQTALLVVLVLLAVALQATEAGPYGANMED...SVCCRDYVRYRLPL 47
1 MQVSTAALAVLLCTMALCNQ.....FSASLAADTPTACCFSYTSRQIPQ 44
48 RVVKHFYWTSDSCPRPGVVLLTFRDKEICADPRVPWVKMILNKLSQ 93
45 NFIADYFETSSQCSKPGVIFLTKRSRQVCADPSEEWVQKYVSDLELSA 92

FIGURE 3

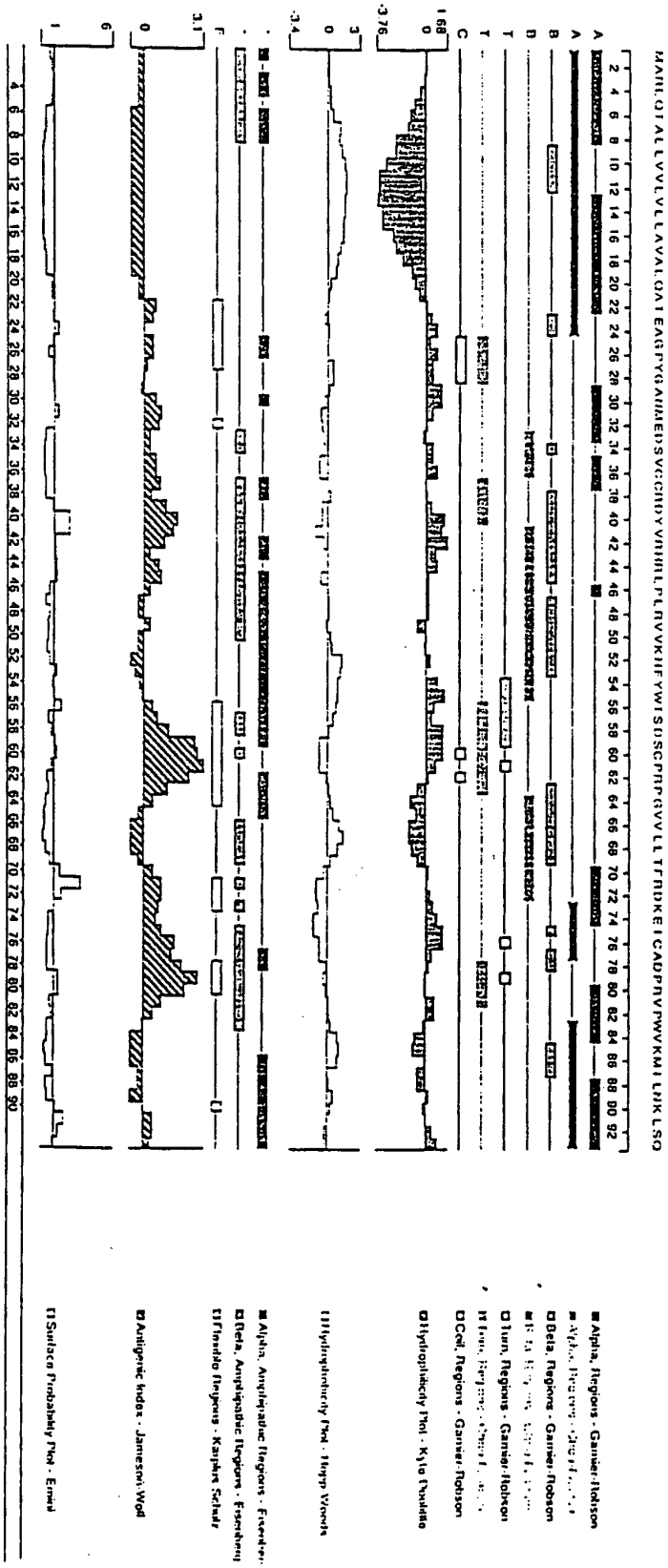
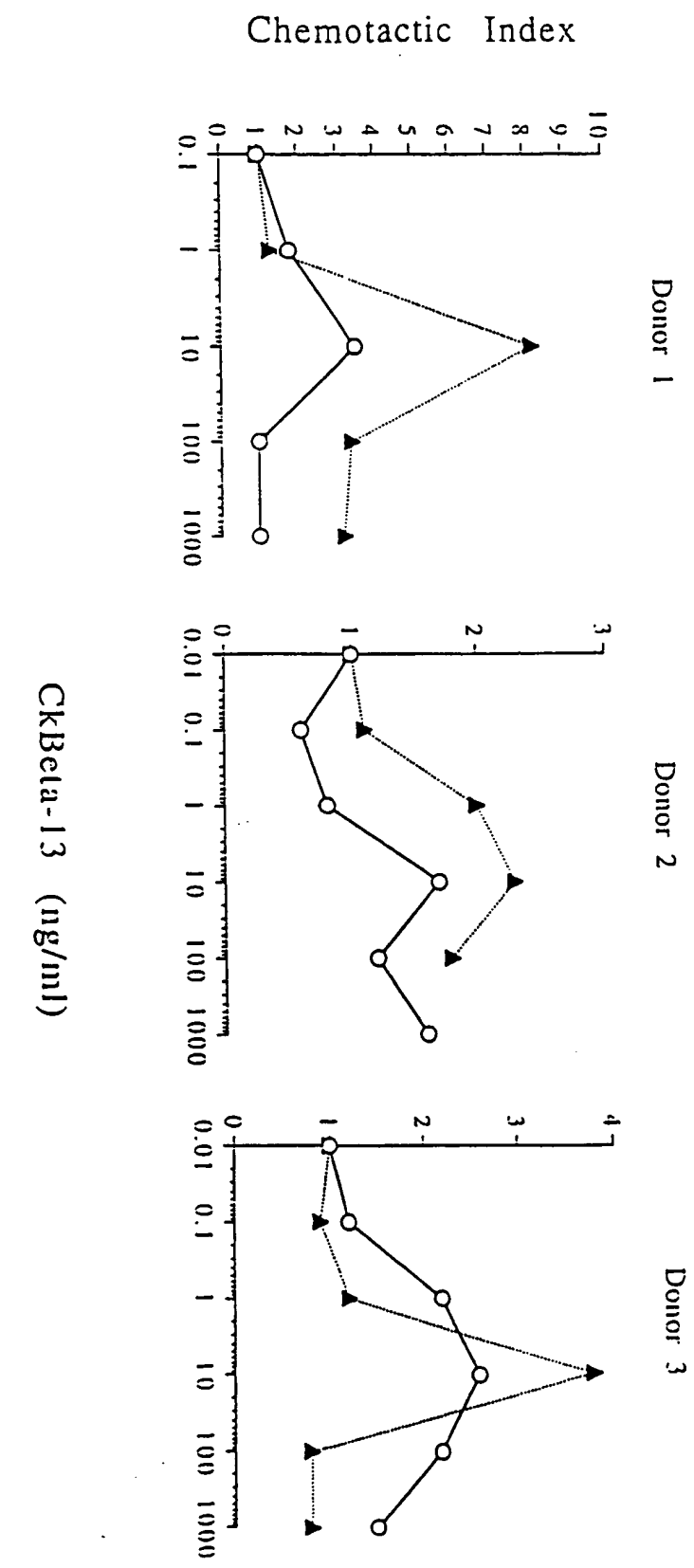


FIGURE 4



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/23144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C12N1/21 C12N5/10 A61K48/00
G01N33/58 C07K16/24 //(C12N1/21,C12R1:19)

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 98 11226 A (SCHERING CORP) 19 March 1998 see page 76 - page 78 see page 81 - page 82; claims	1-20
P,X, L	WO 96 39521 A (HUMAN GENOME SCIENCES INC ;SMITHKLINE BEECHAM CORP (US); LI HAODON) 12 December 1996 L: priority see page 45 - page 46 see page 49 - page 51; claims	1-20
P,X	WO 96 40923 A (ICOS CORP) 19 December 1996 see page 47, line 23 - page 49, line 29 see page 71 see page 86 - page 93; claims	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
22 April 1998	08/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Macchia, G

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 688 927 A (GODISKA RONALD ET AL) 18 November 1997 see Seq.ID:1 see claims	1-20
P,X	CHANG M.-S. ET AL.: "Molecular cloning and functional characterization of a novel CC chemokine, stimulated T cell chemotactic protein (STCP-1) that specifically acts on activated T lymphocytes" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 40, 3 October 1997, pages 25229-25237, XP002062533 see page 25230; figure 1	1-20
P,X	GODISKA R. ET AL.: "Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells" THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 185, no. 9, 5 May 1997, pages 1595-1604, XP002062732 see page 1597, right-hand column; figure 1	1-20
P,X	WO 97 15594 A (SMITHKLINE BEECHAM CORP ;WHITE JOHN R (US); PELUS LOUIS (US); LI H) 1 May 1997 see page 13 see figure 1	18,19
A	SCHALL J.T.: "Biology of the RANTES/SIS cytokine family" CYTOKINE, vol. 3, no. 3, May 1991, pages 165-183, XP002062534	
A	MILLER M.D. ET AL.: "Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines" CRITICAL REVIEWS IN IMMUNOLOGY, vol. 12, no. 1/02, 1992, pages 17-46, XP002050850	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat[illegible] Application No
PCT/US 97/23144

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		EP 0832233 A	01-04-98
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